Effects of Captivity and Testosterone on the Volumes of Four Brain Regions in the Dark-Eyed Junco (*Junco hyemalis*)

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ABSTRACT: **This study investigates the effects of captivity and testosterone treatment on the volumes of brain regions involved in processing visual and spatial information in adult dark-eyed juncos (*Junco hyemalis*). We treated captive and free-living male juncos with either testosterone-filled or empty implants. Captive juncos had a smaller hippocampal formation (HF) (both in absolute volume and relative to telencephalon) than free-living birds, regardless of hormone treatment. Testosterone-treated males (both captive and free-living) had a smaller telencephalon and nucleus rotundus, but not a smaller HF or ectostriatum, than controls. We found that free-living testosterone-treated males had larger home ranges than free-living controls in agreement with earlier experiments, but we found no corresponding difference in HF volume. We discuss the implications of the effect of captivity on HF volume for past and future laboratory experiments.** © 2000 John Wiley & Sons, Inc. J Neurobiol 43: 244–253, 2000

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In most monogamous male songbirds of the temperate zone, plasma testosterone (T) exhibits a short peak early in the breeding season and falls to low levels throughout the rest of the year (Wingfield et al., 1990). Experimental elevation of T in free-living monogamous males during the breeding season alters physiological and behavioral profiles, such that males invest less in parental behavior and more in acquiring mates (Searcy and Wingfield, 1980; Ketterson et al., 1996; Wingfield et al., 1997; Ketterson and Nolan, 1999). For instance, during the breeding season T-treated male dark-eyed juncos (*Junco hyemalis*) (T-males) feed young less often than do sham-treated controls (C-males), and they sing more often (Ketterson et al., 1992; Chandler et al., 1994). Additionally, while their mates are incubating eggs and while young are in the nest, T-males have home ranges that are two to three times larger than those of C-males (Chandler et al., 1994, 1997), which has been shown to result in greater success at extra-pair copulations (Raouf et al., 1997). Although we now know something about the behavioral changes that accompany chronically elevated T levels in the field, we know little or nothing about the influence of these treatments on the neuroanatomy underlying these behaviors. Few experimental studies have attempted to correlate T-induced alterations in behavior with changes in neuroanatomy in free-living birds, in large part because of the logistic challenges associated with field experiments. In the

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present study, we examine possible changes in brain morphology that accompany the increase in home range size triggered by T treatment.

One brain area that is likely involved in navigation through larger home ranges is the avian hippocampal formation (HF). In both birds and mammals the hippocampus plays a crucial role in processing spatial information (O'Keefe and Nadel, 1978; Bingman, 1990) as well as other types of relational information (Wood et al., 1999). Lesioning the HF results in deficits in spatial memory (e.g., Bingman et al., 1988; Sherry and Vaccarino, 1989; Jarrard, 1995), and electrophysiological recordings from the hippocampus in rats have shown that the firing patterns of certain pyramidal neurons in CA1 and CA3 encode the rat's present location in the environment (O'Keefe and Dostrovsky, 1971; Muller et al., 1987). When strains (Rehkämper et al., 1988) or species (Krebs et al., 1989; Sherry et al., 1989, 1993; Jacobs and Sherry, 1992; Spencer, 1994; Reboreda et al., 1996) of animals with superior spatial abilities are compared to closely related strains or species with lesser abilities, the former have larger HFs. Further, in some birds, the development of a larger HF is dependent on experience with spatial memory processing (Clayton and Krebs, 1994; Clayton, 1995; Patel et al., 1997).

This study investigated the effects of T treatment on HF volume in both captive and free-living, dark-eyed juncos. Is the established effect of T on home range size reflected in a larger HF in free-living, T-males during that breeding season? And if this were the case, would this effect also arise in T-treated males kept in captivity? Additionally, we investigated the effects of captivity itself on HF volumes. To verify that T treatment indeed had its expected behavioral effect, we radio-tracked the free-living animals to determine their home range sizes. In addition to the HF, we also measured the total telencephalon volume and two visual system nuclei (n. rotundus and ectostriatum) as control areas that we did not anticipate would be influenced by spatial experience, T treatment, or captivity.

MATERIALS AND METHODS

Subjects: Free-Living Birds

Twenty-two male dark-eyed juncos were captured in the vicinity of the University of Virginia's Mountain Lake Biological Station in the Appalachian Mountains of Western Virginia (37°22′N, 80°31′W) between 30 April and 22 May 1997. Any birds not previously caught and banded were banded with numbered metal leg bands of the U. S. Department of Fish and Wildlife and with a unique combination of colored leg bands. The birds were weighed, and tarsometatarsus length, wing length, and tail length were measured. The first bird was randomly assigned to one of two treatment groups by tossing a coin, and subsequent birds were assigned by alternating them between treatments, until each group contained 11 birds. Each bird was briefly anesthetized with Metofane and implanted with two 12-mm lengths of Silastic tubing (1.47 mm inner diameter, 1.95 mm outer diameter) that were either filled with 10 mm of crystalline testosterone (T implants) or left empty (C implants), and sealed with Silastic glue (Ketterson et al., 1992). Implants were designed to maintain T levels at or near the natural spring peak level for an extended period of time (Ketterson and Nolan, 1992; Chandler et al., 1994). Birds were released back onto their home ranges within 3 daylight hours of capture. Because we were unable to recapture a sufficient number of sham-implanted males, we added two unimplanted free-living males to the control group (henceforth also referred to as C-males). We believe inclusion of nonimplanted males is justified, because we have not detected any behavioral or physiological differences between C-males and unimplanted male juncos in our past research (Ketterson et al., 1996). We were ultimately able to collect both home range data and anatomical data on 5 C-males (3 sham-treated and 2 unimplanted males) and 5 T-males. The other implanted birds could not be recaptured.

Radiotelemetry. After the implanted males had been returned to their territories, we searched for their mates and nests in order to determine their stage of reproduction. Those males found to have active nests were monitored daily, and once the female initiated incubation, we recaptured the male to begin tracking (3 T- and 4 C-males). We tracked two additional males (one C and one T) whose breeding status we did not know and one T-male who was clearly unmated: he sang continuously and was never observed together with a female. In all, we tracked 5 T-males and 5 C-males.

During tracking, males were outfitted with a 0.7-g BD-2A radio transmitter (Holohil Systems Ltd., Corp, OH) attached with a Rappole harness (Rappole and Tipton, 1991) and implanted subcutaneously with an Alzet 1003D osmotic minipump (Alza Corporation, Palo Alto, CA) filled with 10 mg/mL bromodeoxyuridine (BrdU) for a different experiment. After capture, the birds were released where caught and allowed to adjust to the transmitter until the next morning, when we located them using a TRX 1000-S radio receiver (Wildlife Materials Inc., Carbondale, IL). Every location at which a bird was found was tagged with a flag marked with date and time, and the bird's behavior was noted. We waited at least 30 min (so that observations would be independent) and attempted to find the bird again, continuing this procedure over days until we had a minimum of 60 locations for each individual. We then attempted to recapture the bird to remove its transmitter. Birds that were tracked later in the season kept their transmitters on until their final capture, at which time they were perfused.

Approximately 3 weeks after radio-tracking of a bird

began (range of 21–29 days after the day the transmitter was attached and 37–89 days after original implant), it was recaptured, taken to the lab, and weighed; a blood sample was taken for hormone measurement. The birds were then euthanized with an overdose of ketamine (130 mg/kg) and xylazine (7.5 mg/kg) and perfused transcardially with 0.9% NaCl in 0.1*M* Na phosphate buffer (PB) (pH 7.3), followed by 4% paraformaldehyde in 0.1*M* NaPB. The brains were removed from the skull, postfixed for 3–72 hours, and then stored in Na phosphate-buffered saline (PBS). The testes were also removed, weighed, postfixed, and stored in NaPBS.

Home Range Analysis. At the end of the field season, we measured home ranges by recording the distances and angles from each flag to at least one other flag, such that locations for all flags for each bird were in reference to each other. Distances were measured to the nearest centimeter using Sonin Combo Pro Electronic Distance Measurers, and angles were measured relative to magnetic north using a Silva type 80 professional compass. Angles and distances were converted to Cartesian coordinates using a Microsoft Excel spreadsheet and the array of coordinates was analyzed using HomeRange 2.0.1 software (Huber and Bradbury, 1996). We used both the Minimum Convex Polygon method (Stickel, 1954) and the Fourier method (Anderson, 1982) to estimate home range size. The Fourier method was used in two different ways: with a 32 × 32- and a 64 × 64-cell grid. To represent the area in which the bird spent 50 and 95% of its time, respectively, we calculated both MAP(50) and MAP(95) for each grid. A paired *t* test showed no difference between the home range size as estimated by the 64 × 64- or 32 × 32-cell grid method, for either the MAP(50) or the MAP(95), so we report the data only from the 32 × 32-cell grid method.

Subjects: Captive Birds

Thirteen additional male dark-eyed juncos were caught between 27 April and 4 May 1997. They were randomly assigned to a hormone treatment (6 T-males and 7 C-males), implanted as described above within 3 days of capture, and housed in male–male, same-treatment dyads in outdoor flight cages [0.61 m × 1.14 m × 2.44 m (w × d × h)]. They were fed a mix of cracked corn and millet and given regular supplements of mealworms, grated carrots, and hard-boiled eggs. Food and water were available *ad libitum*. Captive birds were randomly matched to free-living birds at the time the latter were outfitted with transmitters and minipumps. At this point, the captives also received a subcutaneous minipump, but no transmitter. Captive birds were perfused at the same time as their free-living counterpart. Birds without a counterpart (or whose counterpart could not be recaptured) were perfused at the end of the experiment. The first captive bird was perfused after 39 days in captivity, the last one after 107 days (median, 78 days).

Hormone Analysis

Immediately before perfusion, blood samples were collected from the brachial vein with heparin-coated microhematocrit tubes. Samples were centrifuged, and the plasma fraction was stored at −20°C. Testosterone assays were performed using a Coat-A-Count Total Testosterone kit (Diagnostics Products, Los Angeles, CA) radioimmunoassay at the Cornell University School of Veterinary Medicine Diagnostics Laboratory. The approximate sensitivity of this assay is 0.02 ng/mL, and intra-assay variation of three plasma pools was 6.7, 6.9, and 14.9%.

Tissue Preparation and Volume Measurements

Brains were incubated in 30% sucrose in 0.1*M* NaPB until they sank and then embedded in 10% gelatin / 30% sucrose. The gelatin blocks were hardened for 2 days in 4% paraformaldehyde and 30% sucrose in 0.1*M* NaPB. They were then cut into 40-μm sections on a freezing microtome. Every third section was mounted on a microscope slide, stained with Cresyl violet, and coverslipped with Permount.

The volumes of the HF, telencephalon (Tel), ectostriatum (Ecto), and nucleus rotundus (Rt) were measured. The criteria used to determine their boundaries were those of Smulders et al. (1995). The sections were either digitized using a high-resolution black-and-white video camera (Cohu, San Diego, CA) connected to an Apple Macintosh IIci computer (Tel, HF, and Rt) or drawn with a drawing mirror and scanned into the computer (Ecto). Because the Ecto was measured at a later date than the other structures, the equipment used for the other structures was not available. The surface area of the structure of interest was measured directly on the computer screen using NIH Image 1.61. We measured the area of every 9th 40-μm section for Tel (for a median of 28 sections measured per brain), every 6th section for HF (28 sections) and Ecto (8 sections), and every 3rd section for Rt (12 sections). These area measurements were multiplied by the sampling interval (0.36 mm for Tel, 0.24 mm for HF and Ecto, and 0.12 mm for Rt) and the resulting volumes were summed to obtain estimates of the total volumes. All structures were measured bilaterally and the sum of the left and the right volumes was used as our final measure in each case.

Statistical Analysis

We used the natural logarithm of all body-size and brain volume measurements for analysis. By chance, the birds that received T implants were slightly smaller at original capture than were those that received C implants (3.8% lower body mass: $F(1,19) = 5.533$, $p = .030$; 2.5% shorter tarsometatarsi: $F(1,20) = 5.157$, $p = .034$). We therefore controlled our analyses of Tel for body-size, using both tarsometatarsus length and a composite measure of size (PC1) as covariates. PC1 was obtained by performing a principal component analysis on all body-size measures

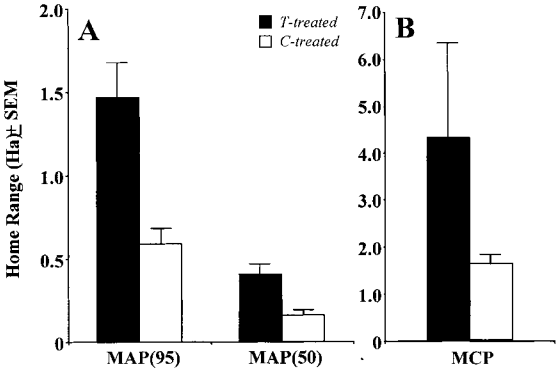

Figure 1 (A) Testosterone-treated males have larger home ranges than control males, as measured by the Fourier method [MAP(95), area in which 95% of the observations fell; MAP(50), area in which 50% of the observations fell]. (B) Home range sizes as measured by the Minimum Convex Polygon (MCP) method do not differ significantly between the two groups, although the magnitude of the treatment effect (T-males having a mean home range size of 2.5 times that of C-males) resembles that seen in the other measures.

(body mass, tarsometatarsus length, tail length, and wing length) and taking the first principal component. The comparisons for HF, Ecto, and Rt were statistically adjusted to compensate for variation in Tel volume. Because HF and Ecto are components of the Tel, their volumes were subtracted from the Tel volume before analysis, such that the Tel volume would be independent of the structure under investigation. We used the general linear model module of Systat 5.2.1 on an Apple Macintosh Centris 610. This analysis technique is a more general version of both a multiple regression and an analysis of covariance, and as such it allowed us to investigate the linear effects of several different independent variables (categorical and continuous) on the dependent variable, while controlling for the other independent variables (Darlington, 1990). For most analyses, we used living condition (captive vs. free-living) and hormone treatment as our main categorical effects, while controlling for continuous variables such as body-size or the volumes of other brain regions. Results were considered significant if $p < .05$.

RESULTS

Home Range Analysis

On average, home ranges for T-males were approximately 2.5 times larger than those of C-males. MAP(95) values were approximately 1.4 ± 0.5 ha for T-males and 0.6 ± 0.24 ha for C-males, whereas MAP(50) values were 0.40 ± 0.15 and 0.17 ± 0.08 ha, respectively [Fig. 1(A)]. There was a significant difference in home range size between T- and C-treated birds [MAP(50): $F(1,8) = 11.879$, $p = .009$; MAP(95): $F(1,8) = 14.033$, $p = .006$]. There was no statistically significant difference between the two treatment groups in home range size as measured by the minimum convex polygon method [$F(1,8) = 1.710$; $p = .227$]. This seems mainly due to the high variability in the T-treated birds, causing a loss of statistical power [Fig. 1(B)]. The mean MCP for T-males was still approximately 2.5 times larger than that for C-males.

Testosterone Titers and Testis Mass

As expected, T-males had higher T titers than did C-males at the time of perfusion [$F(1,21) = 149.517$, $p < .0001$, two-way analysis of variance (ANOVA) with living condition and hormone treatment as main effects]. There were no differences in plasma T between captive and free-living birds, nor was there an interaction between living condition and hormone treatment (Table 1).

There was no difference in testis mass between captive and free-living birds [$F(1,19) = .240$, $p = .630$] or between T-males and C-males [$F(1,19) = 0.107$, $p = .747$], nor was there a significant interaction between the two factors [$F(1,19) = 0.435$, $p = .517$]. The mean mass across all males of both testes combined was 0.244 ± 0.020 g (S.E.M.). A negative correlation between testis mass and perfusion date ($r = -.553$, $p = .006$) indicated that birds perfused later in the season had smaller testes than birds perfused earlier.

Telencephalon Volume

The volume of the telencephalon in T-males was 9% smaller than in C-males [$F(1,19) = 5.064$, $p = .036$; two-way ANOVA with hormone treatment and living condition as main effects], even when tarsometatarsus length or body-size (PC1) was used as a covariate. There was no effect of living condition, nor any interaction between living condition and hormone treatment on telencephalon volume [Fig. 2(B)]. There was no significant correlation between Tel volume and time since implant ($r = -.534$, $p = .091$), nor was there a correlation between telencephalon volume

Table 1 Testosterone Titers (mean ± S.E.M.) in Serum for All Four Treatment Groups.

	T (ng/mL)	C (ng/mL)
Captive	6.46 ± 0.90	0.36 ± 0.19
Free-living	7.21 ± 0.61	0.04 ± 0.03

T: testosterone treated, C: control

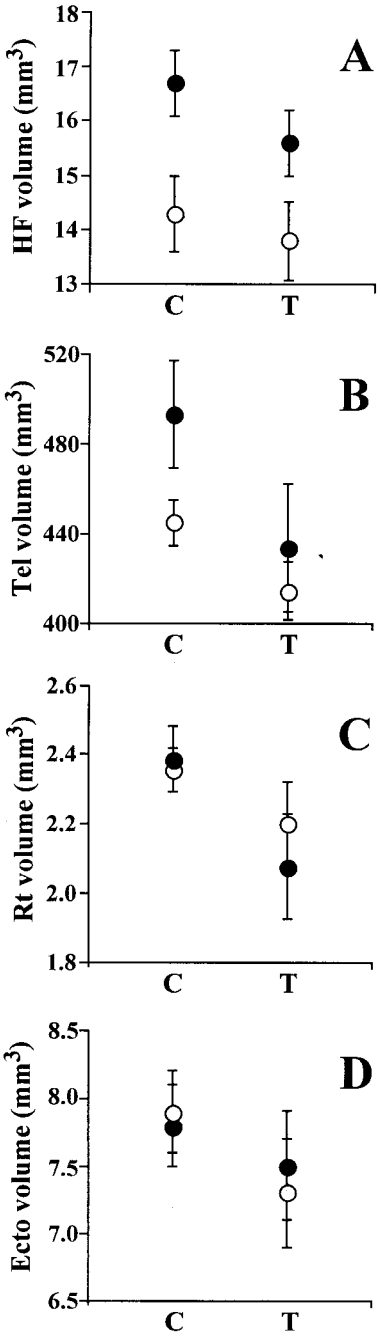

Figure 2 Volumes of HF (A), Tel (B), Rt (C), and Ecto (D) separated according to living condition and hormone treatment. ●, free-living animals; ○, captives. Error bars = S.E.M. All analyses were run using the natural-logarithm transformed data. The significant main effects are as follows: HF is smaller in captive birds than in free-living birds (A), Tel is smaller in T-males than in C-males (B), and Rt is smaller in T-males than in C-males (C). There are no significant effects on Ecto volume (D), nor are there any significant interactions for any of the structures.

and any measure of home range size or any body-size variable, including tarsometatarsus length and PC1.

Rt and Ecto Volumes

Rt volume was positively correlated with total telencephalon volume ($r = .726$, $p < .001$) and, as with the telencephalon, it was 10% smaller in T-males than in C-males [$F(1,19) = 4.857$, $p = .040$; 2-way ANOVA with living condition and hormone treatment as main effects; Fig. 2(C)], even when tarsometatarsus length or body-size (PC1) were used as a covariate. Rt volume, like Tel volume, did not correlate significantly with any body-size measure. Ecto volume was positively correlated with both the non-Ecto portion of telencephalon ($r = .512$, $p = .0125$) and with Rt ($r = .563$, $p = .005$). There was no significant difference between T- and C-males in absolute Ecto volume [$F(1,20) = 2.120$, $p = .161$], although the trend for smaller volumes in T-males was the same as that seen in Tel and Rt [Fig. 2(D)]. There was no difference between free-living and captive birds in the volume of either Rt or Ecto, nor was there an interaction between hormone treatment and living condition.

HF Volume

Absolute HF Volume. There was no significant effect of T treatment on absolute HF volume [$F(1,19) = 1.407$, $p = .250$], nor was there a significant interaction between hormone treatment and living condition [$F(1,19) = .297$, $p = .592$], but HF was 13% smaller in captive than in free-living birds [$F(1,19) = 10.344$, $p = .005$; Fig. 2(A)]. Absolute HF volume of the 13 captives did not correlate significantly with the time they spent in captivity ($r = -0.432$, $p = .141$).

Relative HF volume. HF volume was positively correlated with the volume of the nonhippocampal portion of the telencephalon ($r = .730$, $p < .001$). To determine whether there were changes in HF volume that were independent of changes in Tel volume, we analyzed the variation in HF volume, while controlling for the volume of the nonhippocampal portion of Tel. The analysis used living condition and hormone treatment as independent variables and included all 2- and 3-way interactions in the model. Relative HF volume was 8% smaller in captive than in free-living birds [$F(1,15) = 20.523$, $p < .001$]. For the 13 captives males, there was no significant correlation between relative HF volume and the time they spent in captivity ($r = -.149$, $p = .627$). There was no significant main effect of hormone treatment [$F(1,15) = 1.874$, $p = .191$], nor was there an interaction

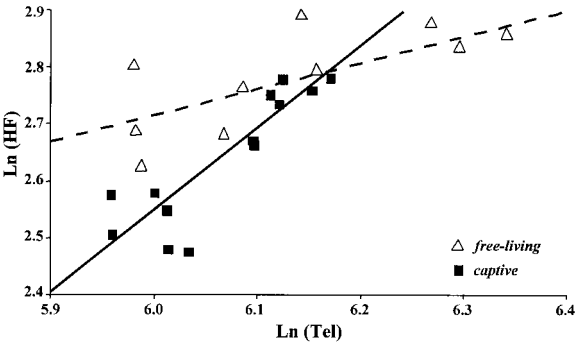

Figure 3 Interaction effect between Tel volume and living condition on HF volume. The slope of the regression of HF volume against Tel volume is steeper for captive (solid line, ■) than for free-living birds (dashed line, △).

between living condition and hormone treatment [$F(1,15) = 1.077$, $p = .316$]. There was a significant main effect of the volume of the nonhippocampal portion of Tel [$F(1,15) = 54.197$, $p < .001$], as well as a significant interaction between Tel and living condition [$F(1,15) = 20.100$, $p < .001$]. Figure 3 illustrates the significant two-way interaction: the slope relating HF to the nonhippocampal portion of the Tel was steeper for the captive birds than for the free-living birds, indicating that any decrease in Tel volume had a greater effect on HF volume in captive birds than it did in free-living birds. The other interactions were not significant.

DISCUSSION

HF was smaller in captive birds than in free-living birds and the relation between HF and Tel volume differed between the two groups. Living condition had no effect on Tel, Ecto, or Rt volume. T-treated birds had a smaller Tel and Rt volume than C-treated birds, regardless of their living condition. Our manipulations of T levels, like those by Chandler et al. (1994), succeeded in modifying home range sizes for the free-living males, but they had no effect on HF volume.

Effect of Captivity on HF Volume

Both absolute HF volume and HF volume relative to the rest of the Tel were smaller in captive birds than in free-living birds. This effect was independent of T treatment, which itself had no effect on HF volume. Captives experienced social circumstances that were different from those of free-living birds. Being housed with another male in a small aviary and being fed daily by humans could be perceived as stressful to juncos and glucocorticoid titers may have been elevated, which could affect hippocampal morphology (Gould et al., 1991; Mizoguchi et al., 1992; Clark et al., 1995). On the other hand, captive birds, which were not subject to the natural stresses of possible predation, territory maintenance, and finding food, probably were better fed and may have had a lower need for the gluconeogenic actions of corticosterone. Indeed, comparing corticosteroid levels of captive juncos (Klukowski et al., 1997) to those of free-living juncos (Schoech et al., 1999), captives have, if anything, lower corticosterone levels. This suggests that the observed effect of captivity on the HF was not likely to be caused by differences in chronic levels of stress hormones.

Birds in small aviaries experience starkly impoverished conditions, which change little over time. Such housing constitutes a form of deprivation (both from spatial information and along many other dimensions of information) that may have been responsible for the decrease in HF volume in the captive birds. In adult mammals as well, behavioral deprivation can have negative effects on many brain structures, ranging from a decrease in number of synapses to an actual decrease in cortical thickness in certain regions (reviewed by Rosenzweig and Bennett, 1996). Decreases in HF volume have also been found in captive white-breasted nuthatches (*Sitta carolinensis*), after less than 2 weeks in captivity (Petersen and Sherry, 1995), whereas captive black-capped chickadees have 50% fewer newly generated neurons in the HF than do free-living conspecifics at the same time of year (Barnea and Nottebohm, 1994). In this light, it is not surprising to find that in juncos as well, captivity had a negative effect on HF volume.

It is also possible that what we observed in the captive juncos was not a decrease in HF volume, but a failure of volume to increase as the breeding season progressed. Captivity of migratory garden warblers (*Sylvia borin*) did not decrease HF volume *per se*; rather, it prevented the increase in relative HF size that occurred in free-living birds during their first migration (Healy et al., 1996). In order to distinguish between a decrease and a failure to increase, we would need to measure HF volumes in free-living birds early in the breeding season, around the time of year at which our captive birds were collected. Regardless of whether HF volume of captive male juncos decreased or failed to increase, the fact remains that captivity had a negative and specific influence on their HF morphology.

In addition to HF being smaller in captives, the relation between HF volume and Tel volume was also

different between the two treatment groups. The slope of the regression relating HF to Tel was much steeper for captives than for free-living birds (Fig. 3). Captive birds with a small brain had a smaller absolute HF than free-living birds with the same brain volume, whereas large-brained birds of both groups had similar absolute HF volumes. Stated differently, HF volume in free-living juncos was not only larger, but also less variable across different Tel volumes, than it was in captive birds. This suggests that conditions in the field may sustain a minimum absolute HF volume, which is achieved regardless of Tel volume. In captivity, in the absence of the demands of living in the field, HF volume is much more closely determined by the volume of the rest of the brain. Determining which aspects of life in the field require absolute HF to be larger would give us a deeper insight into the role and functioning of the HF.

Implications for Laboratory Experiments

The difference in HF volume between captive and wild birds has important implications for studies of spatial memory and other hippocampus-dependent behaviors in a laboratory setting. Our data and those of others suggest that it cannot be taken for granted that after short periods of captivity (possibly as short as 2 weeks; Petersen and Sherry, 1995), birds still have the same neural architecture as they would have had in the field. Similarly, the brain's response to experimental manipulation may be different in free-living and captive birds. Hence, captivity-induced changes in the brain could influence the results of comparative studies in unexpected ways. For example, when comparing the performance of food-hoarding and nonhoarding bird species of the family Paridae (chickadees and titmice) on laboratory tasks requiring spatial memory, it is possible that the effect of captivity on the HF could reduce the food-hoarders' performance on some tasks to the same level as that of nonhoarders. Shettleworth (1995) has reviewed how such comparative studies have yielded variable results in this family of birds.

In the field, food-hoarding birds show an increase in HF volume during the hoarding peak (Smulders et al., 1995), an increase that could be driven directly by decreasing photoperiod, by the experience of hoarding and retrieving large numbers of caches, or by both. Two laboratory studies, however, have not been able to replicate the field data. Decreasing the photoperiod induced molting, as well as food-hoarding behavior in black-capped chickadees (Shettleworth et al., 1995), but had no effect on HF volume (Krebs et al., 1995). Similarly, allowing a month's worth of food-hoarding experience in captivity did not induce a larger HF in willow tits (Cristol, 1996). It is possible that neither study properly manipulated all the variables necessary to replicate the seasonal change observed in the field. Alternatively, captivity may have prevented the expected response of the HF to the experimental manipulations. The implications for studies of HF-dependent behaviors and hippocampal anatomy are clear. Laboratory studies alone may not accurately reflect the actual neural processes that underlie behavior in the wild, and thus field studies may be indispensable in characterizing brain–behavior relations.

HF and Home Range Size

T treatment of free-living males, even though it increased home ranges 2.5-fold relative to C-treated animals, did not have an effect on HF volume. There are two possible explanations. First, home range size as we measured it may not be a valid measure of the birds' actual spatial experience. This is possibly reflected in the fact that there was no significant difference between the home range sizes of C- and T-males when we calculated them using the minimum convex polygon method, which includes every point at which a bird was ever observed. However, the variability in this measure was high for the T-treated group [Fig. 1(B)], such that the lack of significance is difficult to interpret. T-males could also increase their home range sizes without increasing spatial information processing by not storing spatial landmark information about the area in which they look for extra-pair copulations, but instead just remembering the general direction home. Second, home range could be a valid estimate of the birds' spatial experience, but spatial experience may not affect HF volume in this species. Experience with using spatial memory affects the development of the HF in food-hoarding Parids, but not in their nonhoarding relatives (Clayton, 1995). Juncos are nonhoarding birds and their HFs are therefore possibly insensitive to changes in spatial information processing. The question whether natural increases in spatial information processing can influence HF anatomy or function in adult juncos therefore remains unanswered. No experimental manipulation reported to date has been able to increase HF volume beyond its natural range in any species. The only successful manipulations of HF volume have, like ours, decreased its volume from the natural values observed in wild populations (Clayton and Krebs, 1994; Clayton, 1996; Healy et al., 1996).

Effect of T Treatment on Tel and Rt

T-treated birds had a smaller Tel and Rt than C-males, both in the wild and in captivity. Ecto, and to a lesser extent HF, also tended to be smaller in T-males, although the trends were not significant. A similar effect of T treatment on Tel and Rt was found in another sample of T-treated wild juncos from the same site (T. Smulders, D. Enstrom, and D. Sengelaub, unpublished data) and T treatment also reduced Tel size in captive male white-crowned sparrows (*Zonotrichia leucophrys*; Rt was not measured in that study) (Tramontin et al., 2000). Because in the present study T-males were physically smaller than C-males before experimental manipulation, we adjusted brain measures for body-size differences. However, this did not eliminate the significant effect of T treatment on brain volumes. Thus, there appears to be a T-induced decrease in Tel and Rt volumes. At present, we are unclear about why T would reduce the volumes of Tel and Rt.

In castrated male zebra finches (*Taeniopygia guttata*; i.e., with lower T titers) several brain areas, including Rt, are larger than in intact controls (Arnold, 1980). This is consistent with our finding that elevated T titers resulted in a decrease in Rt volume. The zebra finch data were interpreted as reflecting an interaction between the gonadal steroids and the perfusion procedure, producing a nonspecific increase in the volume measures across the brain. If T has a direct effect on Tel and Rt volume, the fact that the HF does not experience this generalized T-induced reduction in volume might be the result of the high levels of aromatase in the songbird HF, which can actively remove T from the tissue by converting it to estradiol (Schlinger, 1997; Saldanha et al., 1998).

Alternatively, T-induced reductions in Tel and Rt volume may be mediated by T's effect on corticosterone titers. T treatment in juncos leads to an increase in corticosterone both in captive and free-living birds (Ketterson et al., 1991; Klukowski et al., 1997; Schoech et al., 1999), and corticosteroids inhibit the growth of the brain in developing rats. For instance, adrenalectomy induces brain growth, whereas corticosteroid replacement suppresses this effect (Devenport and Devenport, 1985; Devenport et al., 1992). In brains that continually replace a certain proportion of neurons, as is the case in songbirds (Goldman and Nottebohm, 1983), such developmental effects may continue into adulthood. If corticosteroid levels explain, in part, the observed effects of T on Tel and Rt volumes in our study, then some parts of the brain, such as the HF, are less sensitive to corticosteroids than others. Testosterone may actually protect hippocampal neurons from the detrimental effects of corticosteroids. In the rat hippocampus, corticosterone causes death of CA3 and CA4 neurons, but only in the absence of testosterone (Mizoguchi et al., 1992), and androgens modulate glucocorticoid receptor mRNA (Kerr et al., 1996) and nerve growth factor (Katoh-Semba et al., 1994). It is unclear whether such potentially neuroprotective effects of T occur in birds and if so, whether they are specific only to brain regions that express androgen receptors such as the HF (Balthazart et al., 1992; Soma et al., 1999).

Although the mechanisms by which T induces decreased Tel and Rt volumes remain unclear, more detailed histology, such as systematic characterization of neuronal turnover rates, may lead to a better understanding of the processes that underlie these anatomical changes.

CONCLUSION

Male dark-eyed juncos that were held in captivity had a smaller HF than free-living birds, and the relation between HF and the rest of the telencephalon differed between the two groups. This finding has important practical implications: results (especially negative results) obtained from experiments dealing with HF-dependent behaviors of birds in captivity may be influenced by abnormal neuroanatomy and may need to be validated by field data. T treatment increased home range size in the free-living birds, but had no effect on HF volume. Thus, either this treatment increases spatial information processing without a concomitant change in HF volume, or the increase in home range occurs without a corresponding increase in spatial information processing. T treatment decreased the volume of the telencephalon and nucleus rotundus in both captive and wild caught birds, but the reasons for this are unknown at present. A better understanding of these anatomical consequences of T treatment will add to our growing knowledge of the advantages and disadvantages of maintaining chronically high T levels and ultimately contribute to our understanding of the evolution of suites of phenotypic traits, controlled by hormones (Ketterson and Nolan, 1999).

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